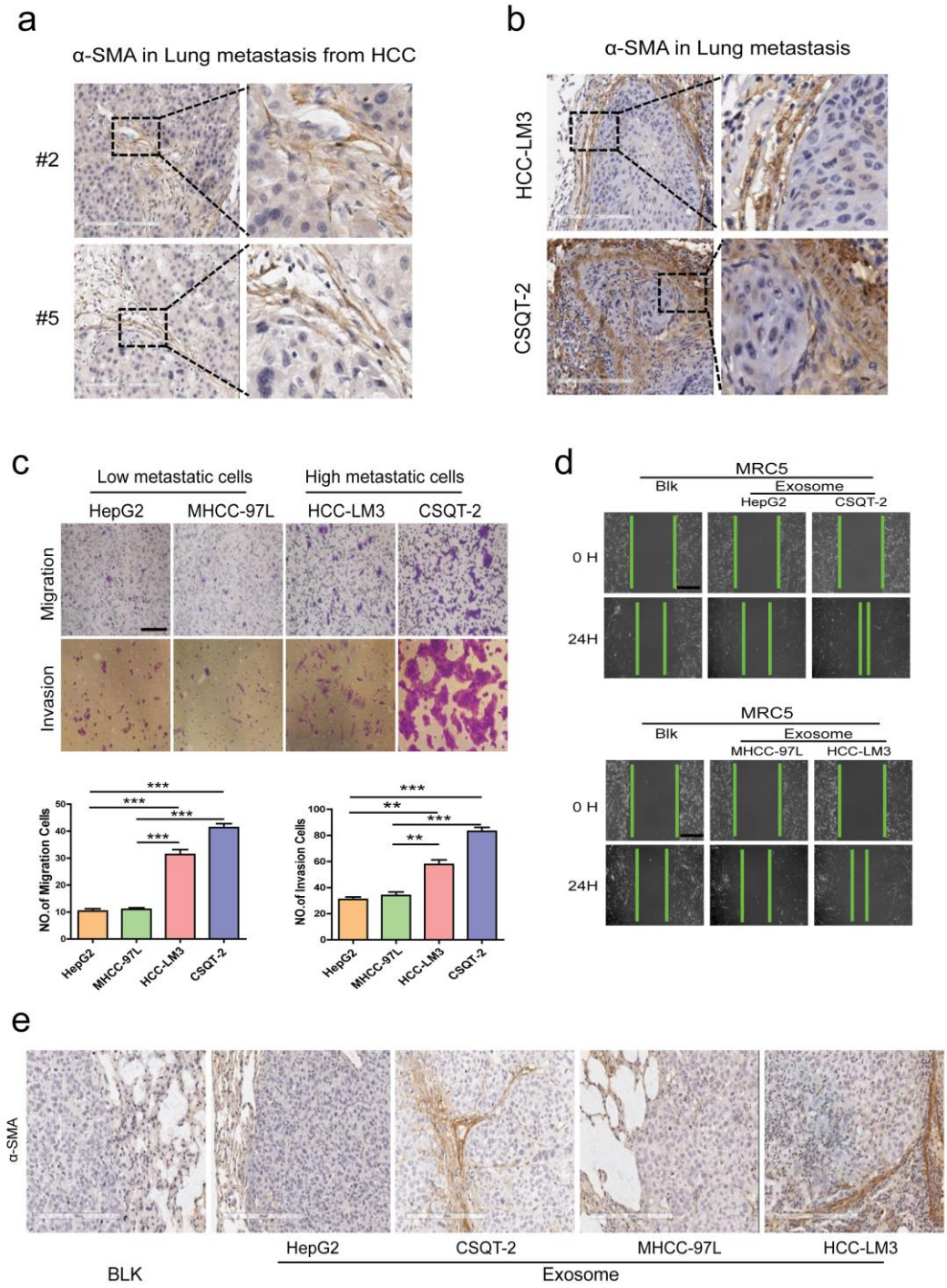
Supplementary Information

Supplementary Figures

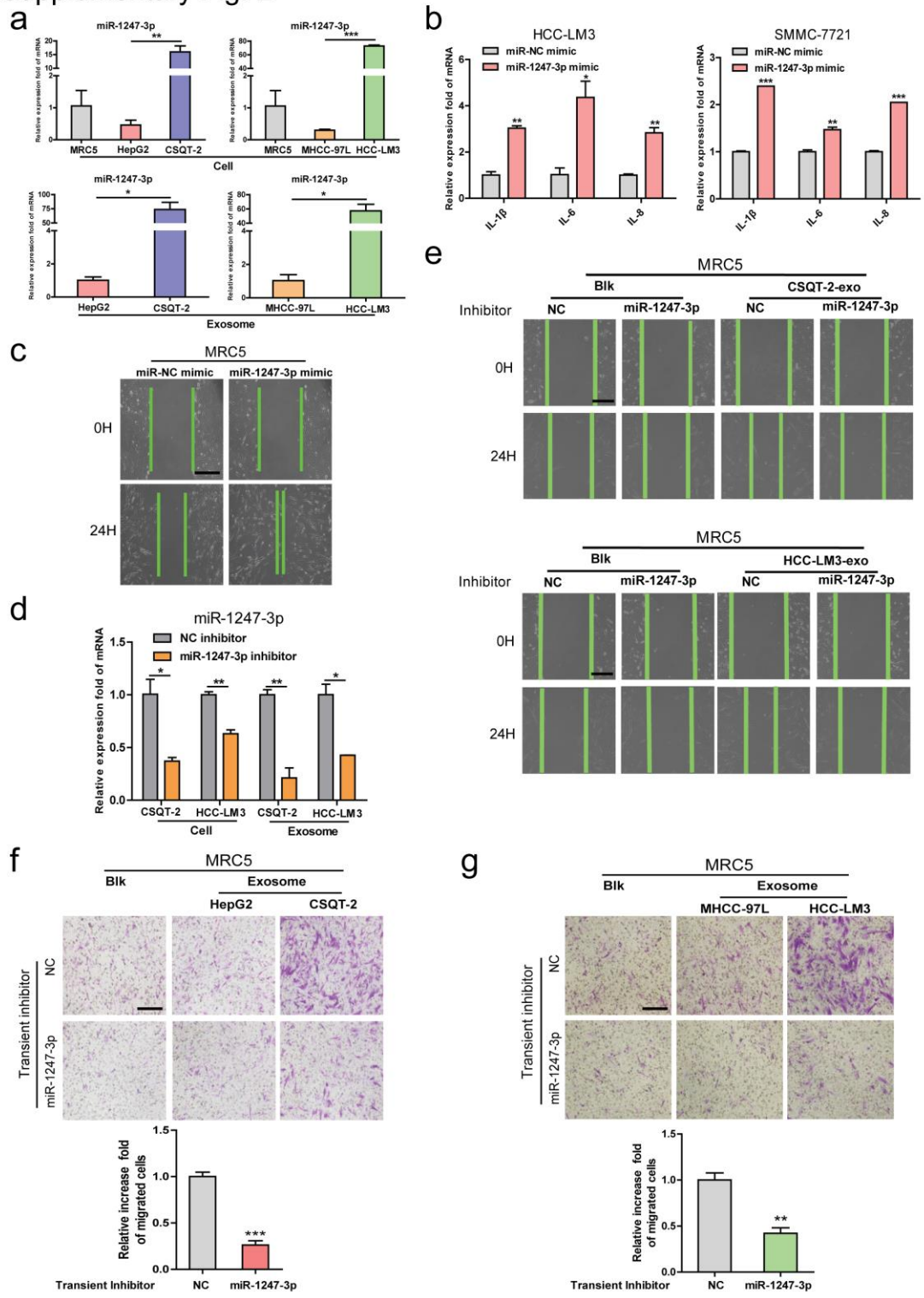
Supplementary Fig. 1



Supplementary Fig. 1 Tumor-derived exosomes activate fibroblasts to foster lung metastasis. **a** Representative images of α-SMA staining in lung metastasis tissues from primary HCC. Scale bar, 200 μm. **b** Representative images of α-SMA staining in lung metastasis tissues from liver cancer cells in mice models. Scale bar, 200 μm. **c** Migration

and invasion assays of different liver cancer cells. Representative images were shown and cells were counted. Scale bar, 150 μm. Data are presented as mean ± s.d. Student's *t* test was used to analyze the data. (**p<0.01; ***p<0.001). **d** Wound-healing assays of MRC5 treated with equal quantities of exosomes derived from different liver cancer cells or blank control. Scale bar, 150 μm. **e** Immunohistochemistry assay of α-SMA staining in lung metastasis tumors in indicated groups. Scale bar, 200 μm.

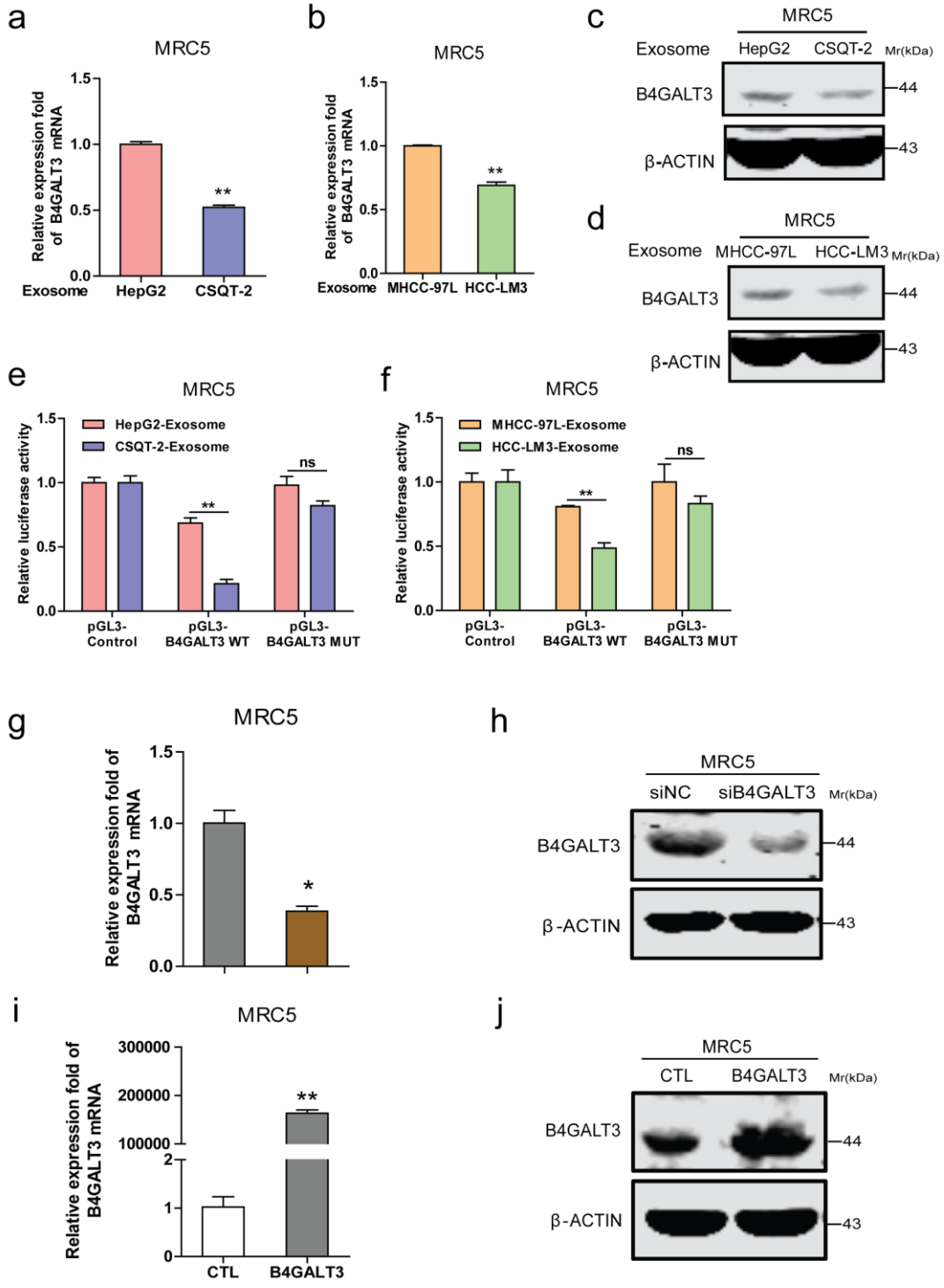
Supplementary Fig. 2



Supplementary Fig. 2 Tumor-derived exosomal miR-1247-3p mediates fibroblasts activation. **a** qRT-PCR of miR-1247-3p in MRC5, HCC cell lines and HCC-derived exosomes. **b** qRT-PCR analysis of pro-inflammatory genes expression of HCC cells transfected with miR-1247-mimic or normal control. **c** Wound-healing assay of MRC5 transfected miR-1247-mimic or normal control. Scale bar, 150 μm. **d** qRT-PCR of miR-1247-3p in highly metastatic HCC cell lines (CSQT-2 and HCC-LM3) and

HCC-derived exosomes stably expressing miR-1247-3p inhibitor or control. **e** Wound-healing assay of MRC5 treated with indicated CM. Scale bar, 150 μm. **f**, **g** Migration assay of MRC5 treated with exosomes derived from HepG2 versus CSQT-2 or MHCC-97L versus HCC-LM3 transiently transfected with miR-1247-3p inhibitor or not. Representative images were shown and migrated cells were counted. Scale bar, 150 μm. Experiments were performed in triplicate and all data are presented as mean ± s.d. Student's *t* test was used to analyze the data. (*p<0.05; **p<0.01; ***p<0.001)

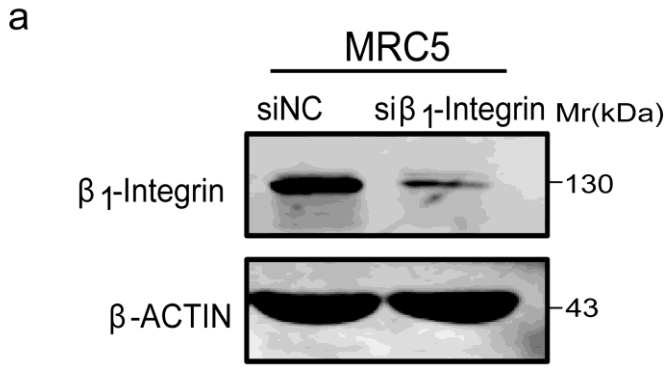
Supplementary Fig. 3



Supplementary Fig. 3 B4GALT3 is the downstream target of highly metastatic cells-derived exosomes in fibroblasts activation. **a**, **b** B4GALT3 mRNA level in MRC5 treated with different tumor exosomes was detected by qRT-PCR analysis. **c**, **d** Immunoblotting assays of B4GALT3 expression in MRC5 treated with different tumor exosomes. **e**, **f** Relative luciferase activity of B4GALT3 in MRC5 treated with different tumor exosomes. **g-j** qRT-PCR and immunoblotting assays of B4GALT3 in MRC5 with

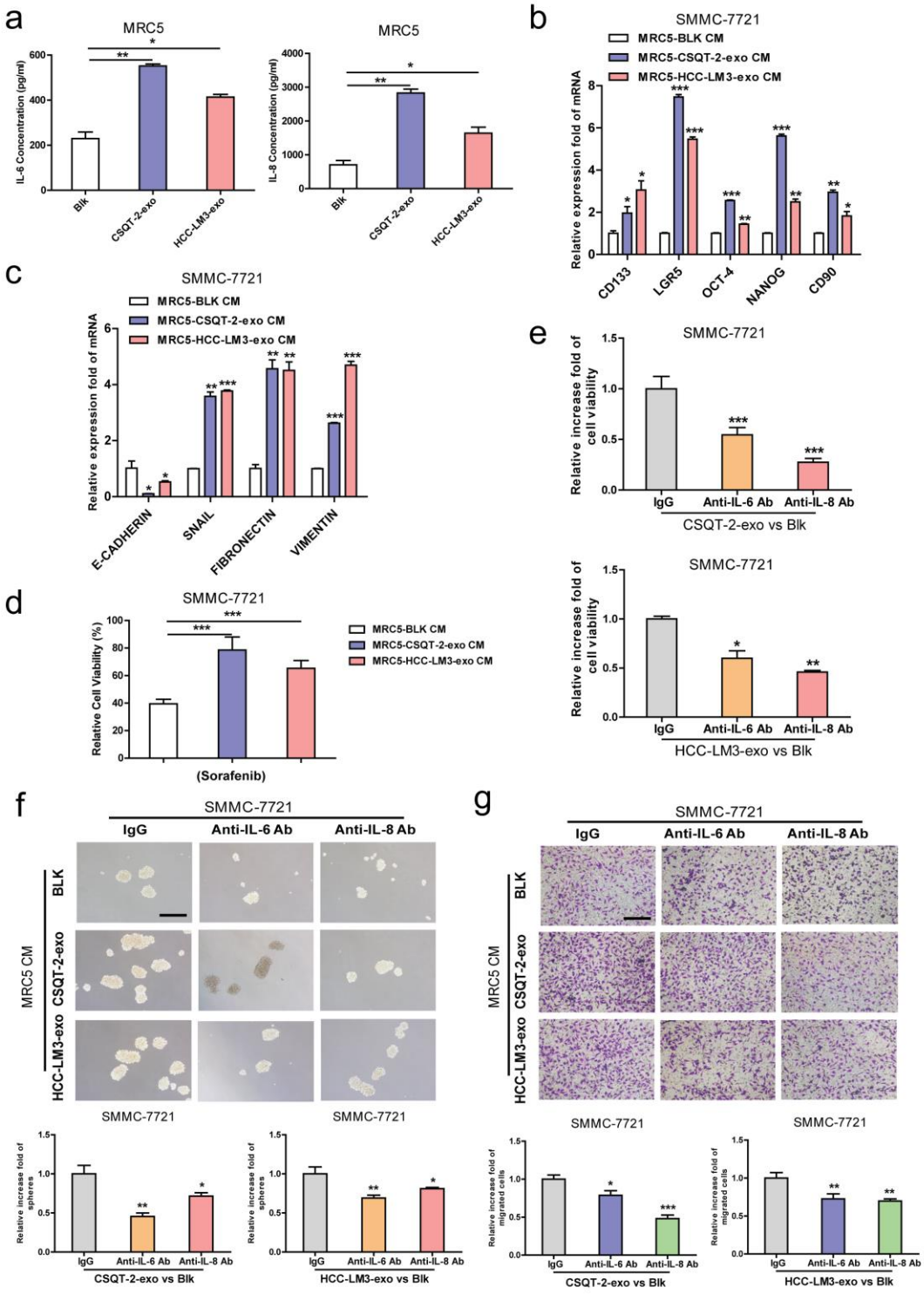
indicated treatments. Results are shown as mean ± s.d. Student's *t* test was used to analyze the data. (*p<0.05; **p<0.01; ***p<0.001)

Supplementary Fig. 4



Supplementary Fig. 4 The effect of β1-integrin knockdown in MRC5. **a** Immunoblotting assays of β1-integrin in MRC5 treated with siRNAs targeting β1-integrin or normal control.

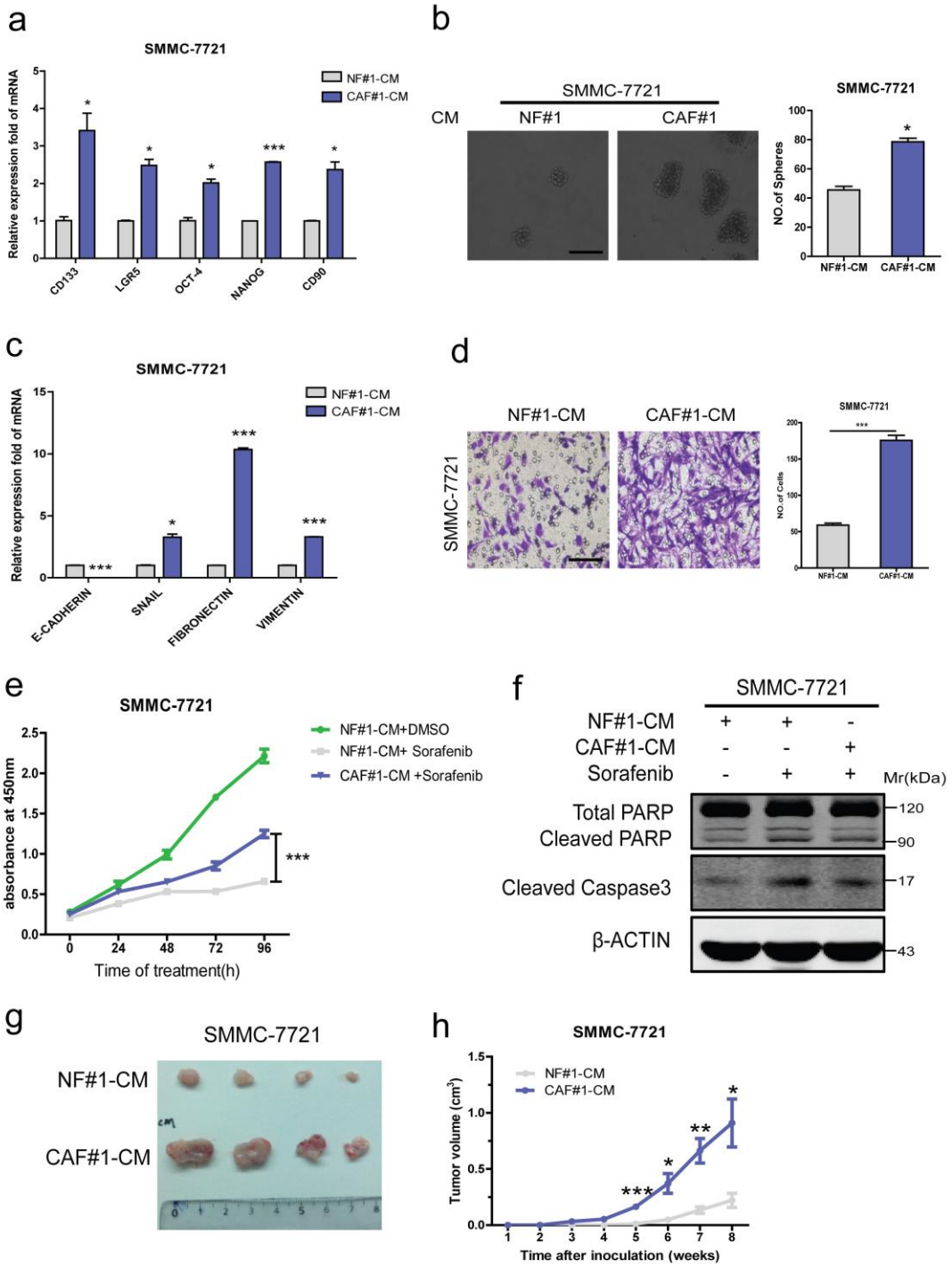
Supplementary Fig. 5



Supplementary Fig. 5 Activated fibroblasts by highly metastatic cells-derived exosomes promote liver cancer progression. **a** IL-6 and IL-8 secretion from MRC5 treated with exosomes derived from highly metastatic HCC cell lines (CSQT-2 and HCC-LM3) or blank control. **b** qRT-PCR analysis of stemness-associated genes expression in SMMC-7721 with indicated treatments. **c** qRT-PCR analysis of EMT-associated genes expression in SMMC-7721 with indicated treatments. **d** Relative cell viabilities of SMMC-7721 treated

with indicated CM in presence of sorafenib. **e** Relative cell viabilities of SMMC-7721 treated with indicated CM containing anti-IL-6/ anti-IL-8 antibody or IgG control antibody in presence of sorafenib. **f** Spheroid formation ability of SMMC-7721 treated with indicated CM containing anti-IL-6/ anti-IL-8 antibody or IgG control antibody. Representative images were shown and spheroid were counted. Scale bar, 150 μm. **g** Migration assay of SMMC-7721 treated with indicated CM containing anti-IL-6/ anti-IL-8 antibody or IgG control antibody. Representative images were shown and migrated cells were counted. Scale bar, 150 μm. Each experiment was performed in triplicate and data are presented as mean ± s.d. Student's *t* test was used to analyze the data. (*p<0.05; **p<0.01; ***p<0.001)

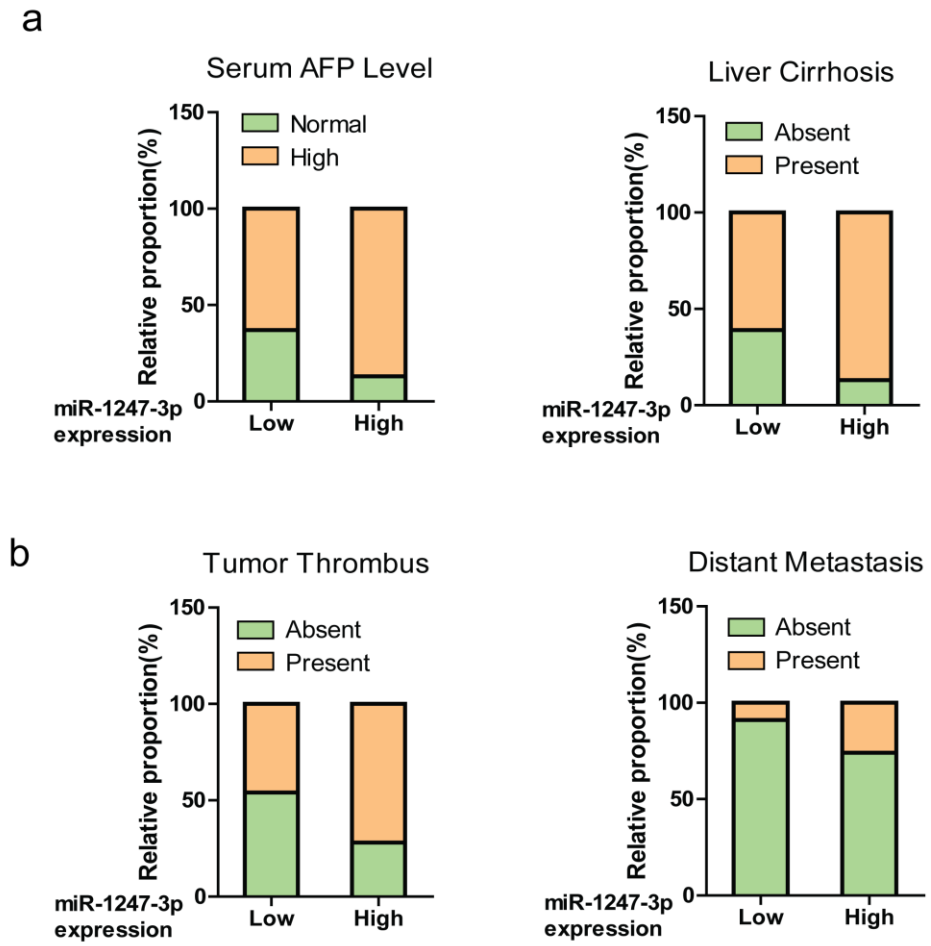
Supplementary Fig. 6



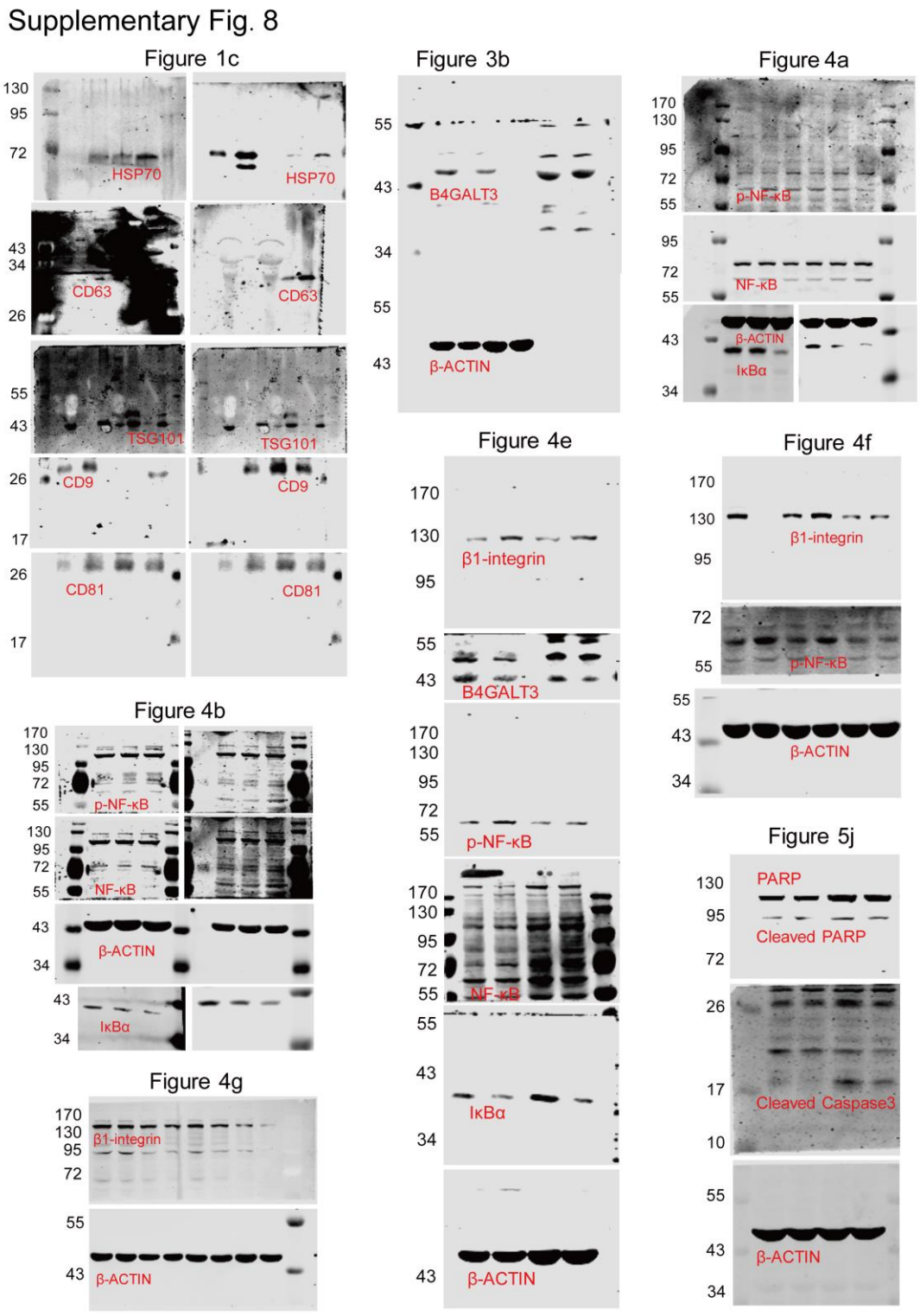
Supplementary Fig. 6 Primary cancer-associated fibroblasts (CAFs) contribute to liver cancer progression. **a**, **c** qRT-PCR analysis of indicated genes expression in SMMC-7721 with indicated treatments. **b**, **d** Spheroid formation and migration assay of SMMC-7721 with indicated treatments. Representative images were represented and spheroid or migrated cells were counted. Scale bar, 150 μm. **e** CCK8 assay of SMMC-7721 with indicated treatments. **f** Western blotting assay of indicated proteins in SMMC-7721 with

indicated treatments. **g**, **h** Xenograft assays of SMMC-7721 with indicated treatments were carried out on nude mice. Representative tumors and tumors growth curves were shown. Data are presented as mean ± s.d. Student's *t* test was used to analyze the data. (*p<0.05; **p<0.01; ***p<0.001)

Supplementary Fig. 7



Supplementary Fig. 7 The correlation of miR-1247-3p expression with clinicopathological features in HCC patients. **a**, **b** High miR-1247-3p expression was correlated with increased AFP level, liver cirrhosis, tumor thrombus, and distant metastasis in 85 HCC patients.



Supplementary Fig. 8 The uncropped scans of western blots from the main figures.

Supplementary Table 1 Summary of Clinicopathologic Variables.

Variables	miR-1247-3p expression (number of cases)		P-value
	Low（n=46）	High（n=39）	
Age			
＜50	27	23	P＞0.05
≥50	19	16	
Gender			
Male	41	33	P＞0.05
Female	5	6	
HBV infection			
Yes	42	34	P＞0.05
No	4	5	
Tumor size			
＜5cm	15	10	P＞0.05
≥5cm	31	29	
Tumor number			
Single	27	15	P＞0.05
Multiple	19	24	
AFP			
＜20ng/ml	17	5	**P＜0.05**
≥20ng/ml	29	34	
Tumor differentiation			
Ⅰ-Ⅱ	13	11	P＞0.05
Ⅲ-Ⅳ	33	28	
Liver cirrhosis			
Yes	28	34	**P＜0.05**
No	18	5	
Tumor thrombus			
Present	21	28	**P＜0.05**
Absent	25	11	
Distant metastasis			
Yes	4	10	**P＜0.05**
No	42	29	

Supplementary Table 2 Sequences of primers and siRNAs.

Gene	Sequences
IL-1β	5'-ATGATGGCTTATTACAGTGGCAA-3' 5'-GTCGGAGATTCGTAGCTGGA-3'
IL-6	5'-ACTCACCTCTTCAGAACGAATTG-3' 5'-CCATCTTTGGAAGGTTCAGGTTG-3'

IL-8	5'-TTTTGCCAAGGAGTGCTAAAGA-3' 5'-AACCCTCTGCACCCAGTTTTC-3'
RAB27A	5'-GCTTTGGGAGACTCTGGTGTA-3' 5'-TCAATGCCCACTGTTGTGATAAA-3'
B4GALT3	5'-CGAGATCAGGGACCGACATTT-3' 5'-GATCGTTCTGGACAGTAGGGC-3'
CD133	5'-GCCACCGCTCTAGATACTGC-3' 5'-TGTTGTGATGGGCTTGTCAT-3'
LGR5	5'-CTCCCAGGTCTGGTGTGTTG-3' 5'-GAGGTCTAGGTAGGAGGTGAAG-3'
NANOG	5'-CATGAGTGTGGATCCAGCTTG-3' 5'-CCTGAATAAGCAGATCCATGG-3'
OCT-4	5'-AGTGAGAGGCAACCTGGAGA-3' 5'-ACACTCGGACCACATCCTTC-3'
CD90	5'-CTAGTGGACCAGAGCCTTCG-3' 5'-GCACGTGCTTCTTTGTCTCA-3'
E-CADHERIN	5'-TGCCCAGAAAATGAAAAAGG-3' 5'-GTGTATGTGGCAATGCGTTC-3'
SNAIL	5'-CCTCCCTGTCAGATGAGGAC-3' 5'-CCAGGCTGAGGTATTCCTTG-3'
FIBRONECTIN	5'-CAGTGGGAGACCTCGAGAAG-3' 5'-TCCCTCGGAACATCAGAAAC-3'
VIMENTIN	5'-GAGAACTTTGCCGTTGAAGC-3' 5'-GCTTCCTGTAGGTGGCAATC-3'
18S	5'-CGGCTACCACATCCAAGGAA-3' 5'-GCTGGAATTACCGCGGCT-3'
si*RAB27A*	5'-GGAGAGGUUUCGUAGCUUA-3'
si*B4GALT3*	5'-CUACUGUCCAGAACGAUCUdTdT-3' 5'-AGAUCGUUCUGGACAGUAGdTdT-3'
si*ITGB1*	5'-CAGCCCAUUUAGCUACAAAdTdT-3' 5'-UUUGUAGCUAAAUGGGCUGdTdT-3'

Supplementary Table 3 Sequences of miRNA mimics.

Mimics	Sequences
hsa-miR-365a-5p	5'-AGGGACUUUUGGGGGCAGAUGUG-3' 5'-CACAUCUGCCCCCAAAAGUCCCU-3'
hsa-miR-4494	5'-CCAGACUGUGGCUGACCAGAGG-3' 5'-CCUCUGGUCAGCCACAGUCUGG-3'
hsa-miR-4513	5'-AGACUGACGGCUGGAGGCCCAU-3' 5'-AUGGGCCUCCAGCCGUCAGUCU-3'
hsa-miR-1247-3p	5'-CCCCGGGAACGUCGAGACUGGAGC-3' 5'-GCUCCAGUCUCGACGUUCCCGGGG-3'
hsa-miR-4688	5'-UAGGGGCAGCAGAGGACCUGGG-3' 5'-CCCAGGUCCUCUGCUGCCCCUA-3'
hsa-miR-4749-5p	5'-UGCGGGGACAGGCCAGGGCAUC-3' 5'-GAUGCCCUGGCCUGUCCCCGCA-3'
hsa-miR-4758-3p	5'-UGCCCCACCUGCUGACCACCCUC-3' 5'-GAGGGUGGUCAGCAGGUGGGGCA-3'
hsa-miR-513a-3p	5'-UAAAUUUCACCUUUCUGAGAAGG-3' 5'-CCUUCUCAGAAAGGUGAAAUUUA-3'
hsa-miR-513b-3p	5'-AAAUGUCACCUUUUUGAGAGGA-3' 5'-UCCUCUCAAAAAGGUGACAUUU-3'
hsa-miR-5684	5'-AACUCUAGCCUGAGCAACAG-3' 5'-CUGUUGCUCAGGCUAGAGUU-3'
hsa-miR-597-3p	5'-UGGUUCUCUUGUGGCUCAAGCGU-3' 5'-ACGCUUGAGCCACAAGAGAACCA-3'
hsa-miR-659-3p	5'-CUUGGUUCAGGGAGGGUCCCCA-3' 5'-UGGGGACCCUCCCUGAACCAAG-3'
hsa-miR-6730-5p	5'-AGAAAGGUGGAGGGGUUGUCAGA-3' 5'-UCUGACAACCCCUCCACCUUUCU-3'
hsa-miR-6754-5p	5'-CCAGGGAGGCUGGUUUGGAGGA-3' 5'-UCCUCCAAACCAGCCUCCCUGG-3'
hsa-miR-6772-5p	5'-UGGGUGUAGGCUGGAGCUGAGG-3' 5'-CCUCAGCUCCAGCCUACACCCA-3'

hsa-miR-6775-3p	5'-AGGCCCUGUCCUCUGCCCCAG-3' 5'-CUGGGGCAGAGGACAGGGCCU-3'
hsa-miR-6796-5p	5'-UUGUGGGGUUGGAGAGCUGGCUG-3' 5'-CAGCCAGCUCUCCAACCCCACAA-3'
hsa-miR-6801-3p	5'-ACCCCUGCCACUCACUGGCC-3' 5'-GGCCAGUGAGUGGCAGGGGU-3'
hsa-miR-6834-3p	5'-UAUGUCCCAUCCCUCCAUCA-3' 5'-UGAUGGAGGGAUGGGACAUA-3'
hsa-miR-6890-5p	5'-CAUGGGGUAGGGCAGAGUAGG-3' 5'-CCUACUCUGCCCUACCCCAUG-3'
hsa-miR-711	5'-GGGACCCAGGGAGAGACGUAAG-3' 5'-CUUACGUCUCUCCCUGGGUCCC-3'
hsa-miR-1247-3p inhibitor	5'-GCUCCAGUCUCGACGUUCCCGGGG-3'